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IN VITRO ANTIBIOTIC SUSCEPTIBILITY STUDIES OF BACTERIA ASSOCIATED WITH KERATITIS.

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Abstract

In vitro tests of the susceptibility of isolates of bacterial keratitis pathogens to antibiotics were carried out in this study. *Staphylococcus aureus* was the most frequently isolated organisms followed by *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Antibiotic sensitivity testing showed a high susceptibility to ciprofloxacin while ampicillin had no effect against any of the isolated organisms suggesting that ciprofloxacin should be considered as the drug of choice in the treatment of keratitis. While single-agent therapy with fluoroquinolones for vision-threatening bacterial keratitis is not advisable, combination therapy with fluoroquinolones and erythromycin offers a reasonable alternative for the treatment of bacterial keratitis. Also, the isolation of *Escherichia coli* shows that further study is required to determine its involvement in keratitis.

1. Introduction

Bacterial keratitis is a suppurative corneal infiltrate and overlying epithelial defect associated with the presence of bacteria on corneal scraping of the eye (Terry *et al.*, 1995; Bourcier *et al.*, 2003). It is a serious complication and the only sight-threatening adverse event that occurs with contact lens wear (Venkata *et al.*, 2002; Holden *et al.*, 2003) requiring recognition, laboratory evaluation and administration of therapy (Liesegang, 1992) being the key to its proper management (Perrigin, 1992). Ulcerative keratitis is among the leading ocular bacterial infections (Jett and Gilmore, 2002) that cause blindness (Niederkorn, 2002) and ocular morbidity (Wong *et al.*, 2003) with increasing evidence of the involvement of bacterial biofilms (Padmaja *et al.*, 2000; Zegan *et al.*, 2002), mucin and other components of tears (Stern, 1991; Aristoteli and Willcox, 2003) and slime envelope (Herbert *et al.*, 1988; Nayak *et al.*, 2001) playing significant roles in their pathogenesis. Paediatric microbial keratitis is a rare but potentially devastating condition similar to adult microbial keratitis, but it is often characterized by a more severe inflammatory response (Stretton *et al.*, 2002).

Some of the factors predisposing a healthy cornea to bacterial keratitis include soft contact lens wear (Galentine *et al.*, 1984; Sharma *et al.*, 2003; Syam *et al.*, 2004), overnight wearing of a rigid contact lens (Sanchez *et al.*, 2001), trauma from several materials, including leaves, branches, dust and stone (Boonpasart *et al.*, 2002). The use of traditional eye medicine and previous viral disease (Wani *et al.*, 2001), lack of hygiene, use of tap water for storing lenses, failure to air-dry lens-storage cases or the use of one-step hydrogen peroxide disinfectant (Houang *et al.*, 2001) are some of the most common risk factors in bacterial keratitis.

Common causes of keratitis are both bacterial and fungal (Laspina *et al.*, 2004) but many bacteria have been more implicated in keratitis. While coagulase-negative staphylococci followed by *Corynebacterium spp.* and *Bacillus spp.* are the most frequently isolated microbes, and pathogenic microorganisms such as gram-negative bacteria are found in only a small percentage of samples (Hovding, 1981; Mowrey-McKee *et al.*, 1992; Hart *et al.*, 1993; Gopinathan *et al.*, 1997; Sun *et al.*, 2004), Boonpasart *et al.* (2002) and Zhang *et al.* (2002) indicted *Fusarium spp, Aspergillus spp and Curvularia spp* as fungal pathogens commonly implicated in bacterial keratitis.

Accurate diagnosis and prompt treatment in form of aggressive antibacterial therapy, preferably with newer generations of antibiotics (Callegan *et al.*, 2003), are the keys to proper and successful management of microbial keratitis (Perrigin, 1992; Fong *et al.*, 2004) since delay in diagnosis and inappropriate treatment could result in a poor final outcome (Periman *et al.*, 2003).

The aim of this work is to examine the in vitro antibiotic sensitivity of bacteria associated with keratitis at the National Eye Center, Kaduna.

2. Materials and Methods

Clinical specimens from keratitis patients, 12 males (70.6 %) and 5 females (29.4 %), presenting at the National Eye Center, Kaduna were investigated for

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bacterial isolates between June 2003 and January 2004. The most prevalent clinical signs observed were chronic keratitis and keratoconjunctivitis, presence of keratitis with severe pain and photophobia, and stromal infiltrates. Using sterile cotton swab sticks, seventeen eye swab specimens were collected for microbiological laboratory evaluation. Used cotton swab sticks were dropped in peptone water and incubated overnight at 37 °C. Microbes that developed overnight were sub-cultured on blood agar, chocolate agar, nutrient agar and triple sugar iron agar and incubated at 35 °C for 24 hours while the chocolate agar was incubated in a candle jar at 35 °C for 24-48 hours. Characterization and identification of isolates were conducted using the Gram's staining method while further identification was conducted using biochemical tests. For the *in-vitro* antibiotic susceptibility tests, each organism was cultured overnight in nutrient broth at 37 °C after which 1ml of these overnight cultures were inoculated into sterile molten agar. The agars were allowed to solidify while sterile forceps was used to aseptically place discs with known antibiotics on already inoculated nutrient agar. The antibiotic discs used were ciprofloxacin (10.0 µg), norfloxacin (10.0 µg), gentamicin (10.0 µg), cloxacillin (5.0 µg), erythromycin (10.0 µg), chloramphenicol (10.0 µg), ampicillin (25.0 µg) and tetracycline (50.0 µg). The plates were then incubated for 24 hours at 35 °C. The zones of inhibition indicating the susceptibility of the organisms to the antibiotics were then observed and measured.

Results

In this study, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were isolated from clinical specimens while culture was unsuccessful in four samples after 48 hours of incubation (Table 1). Blood and chocolate agar plates grew *Staphylococcus aureus* after overnight incubation at 35 °C in CO_2. Eight specimens revealed *Staphylococcus aureus* (41.1 %). Two different specimens revealed *Pseudomonas aeruginosa* (11.8 %) and *Streptococcus pneumoniae* (11.8 %) respectively while *Escherichia coli* (5.9 %) and *Klebsiella pneumoniae* (5.9 %) were isolated each from a specimen. Only one sample was found to contain two different isolates. The lowest percentage occurrence was recorded for *Klebsiella pneumoniae* and *Escherichia coli* while *Staphylococcus aureus* had the highest percentage occurrence. Susceptibility testing showed, in the order of activity against all isolates, that ciprofloxacin was more active than all other antibiotics used as follows: Ciprofloxacin > Norfloxacin > Erythromycin > Gentamicin > Tetracycline > Chloramphenicol > Ampicillin > Cloxacillin. All isolated strains of organisms were sensitive to ciprofloxacin, thirteen isolates were sensitive to norfloxacin, and twelve isolates were sensitive to erythromycin while none was sensitive to ampicillin. Fig. 1 shows the percentages of isolates inhibited by each antibiotic at the concentration used.

Table 1: List of Specimens, Organisms isolated and their antibiotics susceptibility

Specimen no.	Sex	Age (years)	Organisms isolated	Gent	Clox	Amp	Ery	Norf	Cip	Chl	Tetr
NEC01	M	4	No growth								
NEC02	M	Less than 1 year	*Streptococcus pneumoniae*	S	N	N	S	S	S	N	N
NEC03a	M	32	*Staphylococcus aureus*	S	S	N	S	S	S	N	S
NEC03b	M	32	*Escherichia coli*	S	N	N	S	S	S	S	S
NEC04	M	42	*Staphylococcus aureus*	N	S	N	S	S	S	N	N
NEC05	F	40	*Staphylococcus aureus*	N	N	N	S	S	S	N	N
NEC06	M	35	No growth								
NEC07	M	38	*Pseudomonas aeruginosa*	N	N	N	N	S	S	N	N
NEC08	F	13	*Streptococcus pneumoniae*	N	N	N	S	S	S	N	S
NEC09	F	1	*Staphylococcus aureus*	N	N	N	N	S	S	S	S
NEC010	M	50	*Klebsiella pneumoniae*	N	N	N	S	S	S	N	N
NEC011	M	Less than 1 year	*Pseudomonas aeruginosa*	N	N	N	S	S	S	N	N
NEC012	M	15	No growth								
NEC013	M	42	No growth								
NEC014	M	6	*Staphylococcus aureus*	S	S	N	S	S	S	S	S
NEC015	F	Less than 1 year	*Staphylococcus aureus*	N	N	N	S	S	S	S	N
NEC016	F	21	*Staphylococcus aureus*	S	N	N	S	N	S	N	N
NEC017	M	Less than 1 year	Staphylococcus aureus	S	N	N	S	S	S	N	N

Key: Gent = Gentamicin; Clox = Cloxacin; Amp = Ampicillin; Ery = Erythromycin; Norf = Norfloxacin; Chl = Chloramphenicol; Cip = Ciprofloxacin; Tetr = Tetracycline; M = MALE; F = FEMALE

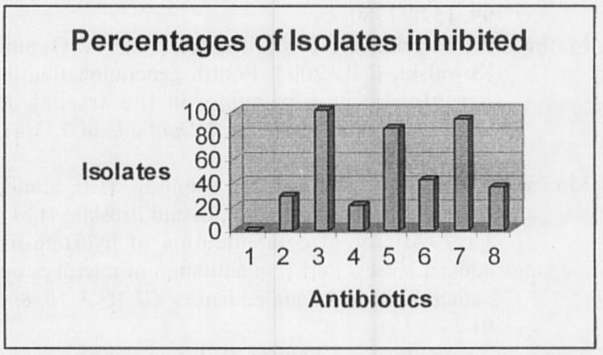

Fig. 1: Percentage of isolates sensitive to antibiotics used

Key:	1 = Ampicillin	2 = Chloramphenicol
	3 = Ciprofloxacin	4 = Cloxacin
	5 = Erythromycin	6 = Gentamicin
	7 = Norfloxacin	8 = Tetracycline

Discussion

Identification of the causative organisms in suspected bacterial keratitis traditionally involves collecting multiple corneal scrapes, which are plated directly onto different solid agar culture media. The involvements of most of the isolated organisms in bacterial keratitis from this study have been reported indicating that, today, they are still relevant in bacterial keratitis. Of the five different species of bacteria isolated, *Staphylococcus aureus* (42.9 %) remains the most commonly isolated organism followed by *Pseudomonas aeruginosa* (14.3 %) and *Streptococcus pneumoniae* (14.3 %) while *Escherichia coli* and *Klebsiella pneumoniae* were the least isolated. The susceptibility pattern of these bacteria to the antibiotics used indicated that, on the average, only a strain of *Staphylococcus aureus* was sensitive to all the antibiotics used with the exception of ampicillin while other strains isolated were sensitive to a minimum of three different antibiotics. The sensitivity pattern observed in *Staphylococcus aureus* may be as a result of development of resistance. However, all strains were sensitive to ciprofloxacin.

From this study, the sensitivity of the isolates to the antibiotics used - ciprofloxacin (100 %), Norfloxacin (92.8 %), Erythromycin (85.7 %), Gentamicin (42.8 %), Tetracycline (35.7 %), Chloramphenicol (28.5 %), Cloxacillin (21.4 %) - indicated that fluoroquinolones remain the best drug of choice in the treatment of ocular infections, with high-effectiveness to all pathogens tested. Although fluoroquinolones have become widely used antibacterial agents in the treatment of ocular infections, with topical, intravitreal and systemic routes of administration being used, a second line agent is needed when resistance is likely, such as in keratitis caused by streptococcal species. Hence, single-agent therapy with fluoroquinolone for vision-threatening bacterial keratitis is not advised. As indicated in this study therefore, keratitis could be treated with quinolones and erythromycin while a combination of aminoglycosides, gentamicin, and erythromycin is sufficient for treating keratitis caused by gram-positive pathogens.

Although the increasing incidence of ciprofloxacin resistance among gram-negative bacilli, especially *Pseudomonas aeruginosa* (Alexandrakis *et al.,* 2000; Diaz *et al.,* 2002) and *Staphylococcus aureus* (Mather *et al.,* 2002), has being reported to occur coincident with increased use of fluoroquinolones (Neuhauser *et al.,* 2003), strains of *Pseudomonas aeruginosa* and *Klebsiella aeruginosa* isolated, on the other hand, were very sensitive to ciprofloxacin and norfloxacin. The *Escherichia coli* isolate was not sensitive to cloxacillin and ampicillin. While the lowered cost and convenience of dispensing a single, commercially available antibiotic such as ciprofloxacin in the initial treatment of bacterial keratitis is desirable, the emergence of ciprofloxacin resistance may be unpredictable. Hence, clinicians should proceed with caution in the initial empiric treatment of bacterial keratitis with ciprofloxacin. Appropriate use of the new fluoroquinolones should be encouraged in order to prevent the rapid emergence and increase of fluoroquinolone-resistant bacteria.

A high percentage of children involved in this study were susceptible to keratitis. This may be ascribed to certain factors such as vitamin A deficiency, malnourishment, poor development or underdevelopment of defense mechanisms and indeed the structure and maturity of the eye itself. This may have also resulted from exposure of children to various traumas, colonization of the eye during birth and childhood diseases such as measles and rubella. Since all isolates from children in this study were sensitive to antibiotics, treatment for children and adult patients with microbial keratitis may follow the same pattern of prescription; topical application of antimicrobial agents initially, followed by application of anti-inflammatory agents. However, with microbial keratitis in children, extra care must be taken to ensure non-toxicity due to blood adsorption.

In conclusion, the involvement of *Escherichia coli* in bacterial keratitis requires further investigation. Also, considering the sensitivity pattern of isolates to the antibiotics used in this study, it is important to note that antibiotic susceptibility tests are necessary to ascertain which antibiotic is best suited for the treatment of bacterial keratitis before prescription is made while a more rational and restricted use of antimicrobials should be adopted to minimize the selection and spread of resistant strains.

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